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THE SIGNIFICANCE OF SCUTE AND PLATE "ABNORMALITIES" IN CHELONIA.

A Contribution to the Evolutionary History of the Chelonian Carapace and Plastron.

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PART II.

IV. The Tail-trunk of Chelydra Serpentina as a Close Approximation to Ancestral Conditions of Carapace and Plastron.

Baur, Hay and others have used as the hypothetical ancestral form an aberrant and perhaps highly specialized Chelonian, *Dermachelys coriacea*, but in the light of the various atavistic recurrences discussed in this paper and in view of the fact that certain definite rows of scutes invariably predominate over others, I have been led to seek elsewhere for primitive conditions.

A closer approximation to the true ancestral conditions is, I believe, to be seen in that portion of the trunk of *Chelydra* that is commonly called the base of the tail. Here if anywhere one would expect to find primitive conditions. The Chelydridæ are generally acknowledged to be our most generalized chelonians,

and it is natural to look for primitive characters in this family, especially in the less specialized regions of the body.

A careful study of the portion of the body posterior to the carapace, which for convenience may be called the "tail-trunk," is fruitful of suggestions. A preparation of the bony structures of this region was made from a very large specimen (Fig. 58) measuring nearly three feet from snout to tip of tail, having a carapace sixteen inches long and a tail-trunk fifteen inches long. The preparation shows the following structures:

There are 33 vertebræ ranging in size from very large bones of nearly a cubic inch displacement to very minute ossicles at the tip of the tail. The first five vertebræ are beneath the carapace and have their dorsal processes in close contact with a longitudinal bony ridge that traverses the last three plates of the neural row of the carapace. These five vertebræ have definite flattened ribs that project laterally about at right angles to the axis of the vertebral column and remind one of the flattened ribs in the carapace of *Dermochelys*. The first and second of these ribs are very large and articulate by means of enlarged heads with the proximal ends of the ileum. These specialized vertebræ and ribs form the sacrum (Fig. 58, 1 and 2).

Surmounting the dorsal processes of the eighth to the fifteenth vertebra is a row of six large bony tubercles (*t.* 1 to 6) that have no reference to individual vertebræ. The dorsal processes of the latter are not the centers of ossification for the tubercles and there is no articulation or fusion between the two series of structures. Posterior to the sixth bony tubercle are fourteen tubercles (*y.* 1–14), with either membraneous cores or no cores at all, ranging in size from structures almost as large as the sixth bony tubercle to extremely small scales with dorsal ridges. Anterior to the first bony tubercle are three small soft tubercles (*x*) occurring at intervals of about half an inch, and anterior to these we find the two procaudal (*pr.* 1 and 2) and the single pygal plate (*p*) of the carapace, overlying the first four vertebræ. We have then the following heterogeneous series of structures: two procaudals, one pygal, three small soft tubercles, six large tubercles with bony cores and finally a graduated series of 14 tubercles merging into ridged scales. It seems reasonable to suppose

that all these structures of the dorsal row, whether scutes with bony cores, scutes without bony cores, or bony plates that have been separated from their original scute coverings, are essentially homogeneous, and that the differences seen in the different regions are secondary or perhaps tertiary modifications. It seems probable that the scutes at the posterior end of the row represent the most primitive condition, which, through a process of continuous variation have become more tubercular in form and have acquired bony cores by the gradual ossification of membraneous tissue. The three soft tubercles surmounting the fifth to seventh vertebræ probably represent a reduced condition in adaptation to the fact that the base of the tail requires flexibility and must be swung from side to side or partially withdrawn under the carapace. The presence of such large prominences as the bony tubercles would seriously interfere with the mobility of the tail. The procaudal plates seem to have been the last of the neural tubercles to have been flattened out to form the dermal carapace. The last neural scute is probably the original chitinous sheath of one of the procaudals, doubtless the second one. The scute of the first procaudal I believe to have been crowded out in the process of scute reduction that will be discussed later.

Over the 33 vertebræ there occur 27 structures of homogeneous origin, that may be designated scutes. This number is sufficiently at variance with the number of vertebræ to preclude the possibility that they have had a segmental arrangement. Moreover, their irregular arrangement and the dermal origin of the bony cores make it certain that they are independent structures, in opposition to views expressed by Gadow and others.

An examination of the entire tail-trunk of another large specimen revealed the rather striking fact that the number of principal rows of tubercles and large scutes in this region is identical with that of the carapace and plastron, and that smaller, less regular rows of tubercles and scales represent the principal lost rows. A section of the tail-trunk was slit down the median ventral line and flattened out for convenience in drawing, and from this the somewhat diagrammatic Fig. 55 was constructed. I was able with certainty to homologize seven principal rows, three dorsal, two

lateral and two ventral, and have named them after their homologues in the carapace and plastron, neurals (*N*), costals (*C*), marginals (*M*), and plastrals (*P*). Smaller and less regular rows of tubercles and scales are homologized with the secondary and lost rows as follows : inframarginals (*IM*), supramarginals (*SM*), interplastrals (*IP*), and neuro-costals (*NC*), a row not occurring in any modern species. In the following more detailed description the above names will be used for the rows concerned.

The costals are large scutes with a marked tendency toward dorsi-ventral flattening. Their apices are directed posteriorly and the growing point is thus situated near the posterior margin of the scute, a fact that causes the scute to grow anteriorly and laterally and very little posteriorly. The marginals are as large as the costals and are flattened so as to expose two surfaces, a dorsal and a ventral. The apex is directed away from the axis of the body and the growth of the scute is principally inward and slightly forward. The plastrals are paired, large, flat and nearly rectangular. They suggest by their appearance the plastron scutes. Their growth is inward and forward, as is that of their homologues in the plastron.

The two secondary dorsal rows, supramarginals and neuro-costals, consist anteriorly of small tubercles that fade out posteriorly into small irregular flat scales. The inframarginals are not tubercular but are rather large and fairly regular flat scutes. The interplastrals are diamond-shaped scutes forming a discontinuous row and occupying the angles made by four plastrals. This row is sometimes entirely absent on the tail-trunk of *Chelydra*.

It is clear then that in the tail and tail-trunk of *Chelydra* we have seven principal rows of scutes and between each pair of principal rows a secondary row, less regular and far less prominent. This would give a total of fourteen rows, of which seven are primary and seven secondary. Supposing that the carapace was originally continuous with the tail-trunk, we must imagine a gradual suppression of the secondary rows by the primary ones, until in the most highly specialized condition, seen in the terrestrial Emydidæ, only the seven primary rows have survived.

The order of loss of the seven secondary rows may be con-

jectured from an examination of the directions of encroachment of the principal rows upon the secondary rows. On this basis one might give the order of loss as follows: first, neuro-costals, which on account of inward encroachment of the costals and the outward encroachment of the neurals, would receive the severest pressure; second, the supramarginals located between the inwardly-encroaching marginals and the outwardly-encroaching costals; third, the interplastrals, between the two inwardly-encroaching plastrals, but occasionally able to escape pressure by occupying angles between four plastrals; fourth and last, the inframarginals, which were subject to pressure only on one side — that of the marginals — and were in addition required in more primitive forms with small plastrons to help bridge the space between the carapace and plastron. With the great increase in the size of the plastron which has taken place in higher forms, the space allotted to inframarginals became more and more contracted until they were crowded out entirely.

Does this order of loss correspond to any facts in nature? The same conclusion as to order of loss, I believe would be reached if we based our results on the prevalence, scarcity or absence of these rows as normal structures in existing species. No trace of neuro-costal scutes are found. Only one species, *Macrochelys temmincki*, possesses supramarginals. Interplastrals occur as isolated median ventral scutes in several families. Inframarginals occur normally in all the more primitive families and as axillaries and inguinals in all but the most specialized terrestrial forms.

This condition as derived from the tail-trunk of *Chelydra* differs rather radically from the ancestral condition derived from *Dermochelys*, in which there are twelve rows of equal rank. I am inclined to believe that *Dermochelys* is an extremely aberrant type with only a most distant connection with the phylogenetic line of *Chelonia*. The *twelve* keels of *Dermochelys* are comparable, I believe, to the *seven* keels of modern forms, and the irregular rows of plates and scutes between the keels are comparable to the secondary rows of scutes seen in the tail-trunk of *Chelydra* and represented in the carapace and plastron by inframarginals, supramarginals, interplastrals and neuro-caudals.

V. Further Evidence of the Former Existence of a Dermal Carapace in Chelonia, as Derived from Specimens of Graptemys.

The position just taken rests on the assumption that the carapace and plastron were at one time continuous with that portion of the trunk just posterior to them and that the carapace and plastron have undergone a gradual process of specialization that has caused them to depart widely from ancestral conditions. The tail-trunk would then preserve to a greater or less extent its original character.

On this assumption, then, there once existed a complete row of dermal tubercular ossicles overlying the vertebræ. That certain ancient forms did actually possess these ossicles was shown by Hay in the case of *Toxochelys serrifer*, but the question arises whether or not we have sufficient evidence that *Toxochelys* represents the ancestral condition of our modern forms.

For a long time I looked for definite traces, other than the keels, of such ossicles as are seen in *Toxochelys*, but met with no success until I had nearly completed the present paper. Then by merest chance I stumbled on the evidence needed to clinch the argument.

I had kept alive a few specimens of *Graptemys* in a small aquarium, but one by one they sickened and died, with one exception. Their death was doubtless due to the fact that this species is highly specialized in its diet, feeding exclusively on a species of viviparous gastropod that is abundant in Lake Maxinkuckee. They never learn to use other food, and, in lack of their special diet, starve themselves to death.

The surviving specimen was a nearly adult female that had been kept on account of its many peculiarities. After eleven months of captivity it was killed and examined for plate abnormalities. This examination revealed the presence of several small, loose, ossicles that were inlaid, as it were, in the bone of the neural plates and were situated beneath the keels of the second, third, fourth and fifth neural scales, *i. e.*, exactly in the positions occupied by the ossicles found in *Toxochelys*. The largest of these ossicles (see Fig. 5) was situated beneath the keel of the third scute and extended partially under the anterior

margin of the fourth scute. The fifth scute is a supernumerary neural (No. 8) and hence the occurrence of an ossicle at its keel shows that it belongs in the neural row in spite of the fact that it is crowded to one side. All of the ossicles are imbedded or inlaid in the centers of certain of the neural plates. If they were merely the loosened centers of these plates, we would surely expect to find them on all the plates instead of just those which lie beneath the keels of the scutes. In what respect do these ossicles, then, differ from those seen in *Toxochelys serrifer* by Hay? Merely in this, that they are much reduced in size, so that each is confined to one neural plate, extending back so as to overlap the anterior portion of another plate.

The specimen is an oddity in many respects. It is unusually long in the carapace; possesses fifteen plates in the neural row instead of the normal number, twelve (three of these probably representing supernumerary procaudals); two extra costal plates or ribs, one quite vestigial; two supernumerary scutes of large size; one supernumerary costal scute of large size on the right side; and two well-developed supernumerary inframarginals on each side. Other minor peculiarities might be noted, but they do not concern the carapace or plastron. All of the anomalies mentioned may be viewed as of atavistic character, and it should not be surprising to find that the curious specimen shows an even more significant atavistic recurrence than any other specimen thus far examined, namely, a reversion to the condition seen in *Toxochelys*. That the genus *Graptemys* originally possessed a median dorsal keel composed of prominent bony tubercles covered with chitinous sheaths (scutes) is rendered extremely probable when we examine the young, especially that of *G. pseudogeographica*, a specimen of which is pictured in Agassiz' Contributions to the Natural History of the United States, Vol. II., Plate II., Figs. 11 and 12. This specimen, which may perhaps be an extreme type, although Agassiz does not suggest that such is the case, shows a series of three very remarkable dorsal tubercles on the second, third and fourth scutes. These tubercles furnish a close approximation in general form to those seen on the tail of *Chelydra*. It will be noted that these tubercles occur exactly in the places where I have found the vestigial ossicles in a specimen

of *Graptemys geographica*, and that the most prominent tubercle is that on the third scute, a fact that is of interest in view of the larger size of the vestigial ossicle on that scute.

The adult of *Graptemys pseudogeographica* retains decided traces of these tubercles throughout life and their location is marked by dark blots of pigment that in later life form the only prominent color-markings of the animal. The discovery of vestigial ossicles in *Graptemys geographica* led me to investigate a very large female specimen of *Graptemys pseudogeographica*, that has been in confinement, and, like its relative, starving for many months. Immediately beneath the dark blots of pigment on the neural scutes there are on this specimen thin, scale-like discs of bone with a looser and less dense texture than the underlying bony plates. I have been able to examine but one adult specimen of *Graptemys pseudogeographica*, but this one appears to be perfectly normal in every other respect. It is to be noted that the vestigial plates in the last-mentioned specimen occupy exactly similar positions, with respect to scutes and plates, as do the vestigial ossicles in the anomalous specimen of *Graptemys geographica*, described and pictured above (Fig. 5). The probable explanation of both these conditions is that long-continued starvation has brought about a resorption of the portions that united these ossicles with the underlying neurals. That bone is resorbed either by normal processes or as the result of pathological conditions I have observed in several cases where holes have been eaten entirely through the bone of the carapace, as the result of starvation. Only a thin cap covering the top of the tubercle is of dermal origin, the main portion of the prominence being merely an outgrowth of the periosteum of the neural process. Examinations of developmental stages have revealed no discontinuity between the cap and the rest of the keel, but the microscope reveals the difference in histological structure between the cap and the underlying bony plate.

That tubercular ossicles existed over the neural processes of other ancient reptiles is shown by the fossil *Stegosaurus*. Here the dermal processes are very large and prominent and are much fewer in number than the neural processes that underlie them. This points to the entire independence of dermal bones and the vertebræ, and hence to the non-segmental character of the dermal plates.

VI. The Color Pattern of Chelonia as Confirmatory Evidence for the Former Existence of a Dermal Armor.

Evidence is not lacking that points to an original striped condition of the chelonian carapace. The neck and tail of most tortoises show characteristic stripes which on careful examination may be analyzed into rows of scales with similar coloration. When the scales are large enough, it will be seen that each has a center of pigmentation coinciding with its center of growth. Now the coloration of the carapace and plastron is nothing more than a series of scales or scutes, each with its pigmental center. The striped effect is lost through the great increase in the size of the scute and the consequent separation of the centers of pigmentation.

The pigmentation of scutes is typically concentric in character, whether the pattern consist, as in *Chelydra* and *Aromochelys*, of radiating bands of pigment having their center located at the center of scute growth, or concentric rings as in *Graptemys*, or lastly, of a light area occupying the center of growth, and all the rest solidly colored, as in *Clemnys guttatus*.

Frequently a great complexity of marking arises through the secondary complications of primary markings, but these conditions are seen in a simplified condition in the developmental stages.

It strikes one very forcibly that there is an intimate relation between scute growth and pigment distribution. The two processes have a common center and go hand in hand. The *areola*, or egg-plate (see Fig. 57, dotted lines) forms a convenient locus for measuring both processes. This location corresponds, curiously enough, with the keel of the scute and hence with the center of dermal ossification.

An examination of embryos of *Chelydra* shows that the coloration consists of dark patches of melanin pigment at the tip of the tubercular processes of the keels. The marginals are marked with small black spots at the posterior edge of each scute, sometimes running back over the anterior margin of the next scute. Specimens of *Chelydra* a year or two old have a radiating pattern with the center of pigment proliferation at the keel. In older specimens a solid coloration obscures everything.

Older embryos of *Graptemys* have, as the first indication of pig-

ment, one dark spot at the median posterior margin of each scute, *i. e.*, at the keel (see Fig. 56). Later on two or more other spots of a similar appearance are produced in very definite positions on the various scutes. These secondary spots never become quite so prominent as the primary ones, but continue to develop like the latter into concentric ocellated markings (Fig. 57). The rings that constitute these ocellated spots are formed by repeated splitting of the innermost ring of pigment, in brief are proliferated from a center of pigment deposit. After three or four cencentric rings have been laid down, a well-marked unpigmented band appears on the periphery of the whole spot. It is this light band that produces the most pronounced secondary complexity, for it sends out processes to neighboring spots and forms the characteristic reticulated pattern that has given the name " map-turtle " to the species. In the marginals the pattern is not so complex, since no secondary spots appear. The concentric rings of the primary spot, however, often take on fantastic shapes that all but obscure the original unity of the coloration. It is very interesting to note that the spots in the marginal series are found as a rule half on one scute and half on the next, so that the light peripheral band that separates adjacent spots frequently coincides with the sutures of the dermal plates beneath. The spots then occupy the growth centers of the plates and no longer hold any close relationship with the scutes. *Pigmentation seems to be intimately connected with dermal ossification.* The scutes must have grown away from their original positions, thus overlapping the sutures of the plates, and lending strength to the general structure. The direction of movement has been forward, as is found by examination of the first pair of marginal scutes that run over upon the nuchal plate. The original cause of the forward movement was, I believe, the increase in size of the nuchal plate which must have pushed back the marginal plates. The scutes would of course occupy their original positions or would continue to crowd the nuchal scute into a still smaller space.

That the primary ocellated spots denote centers of dermal ossification will, I believe, be admitted. What then is the significance of secondary ocellated spots, which have an exactly similar appearance and method of development? They must, I

believe, represent centers of dermal ossification and hence the location of scutes that formerly occupied the position now occupied by the spots. Evidences that serve to increase the probability of this conclusion may be adduced:

1. Whenever supernumerary scutes recur each has at its growth center an ocellated spot.

2. These supernumerary scutes come in at places where normal specimens have definitely placed secondary ocellated spots.

3. There are never any secondary ocellated markings on the marginal scutes, which agrees with our idea that the marginal rows contain nearly their original number of scutes and hence we would not expect to see traces of lost scutes in these rows.

4. The light bands that form the reticulated pattern of adult *Graptemys* have often the exact shapes of existing dermal plates. This is particularly the case in the procaudal region.

5. Considering the light bands as original scute boundaries, we can count ten costal scute areas in *Graptemys* or *Trachemys*.

6. On the neural scutes of *Graptemys* there are several much smaller ocellated spots that lie near the outer edges of the scute. These spots, I believe, are the vestiges of the small scutes that I have earlier designated as neuro-costals, and that were the earliest rows of scutes to be crowded out (Fig. 57). Four or more well-developed spots occur close to the marginals on the costals and occupy positions similar to the supramarginal scutes of *Macrochelys temmincki*.

At the angles made by adjacent neurals and costals occur ocellated spots that have the appearance of having been squeezed out between two scutes. These I believe to be the vestiges of the lost neurals and costals (see Fig. 57). When the lost scutes recur they have these spots at their growth centers.

In the bridge region of *Graptemys* and *Chrysemys* a confused series of dark colorations appears that seems to have no reference to any existing structures. But when the inframarginals recur, we find that these apparently meaningless markings fall into place as the spots of this lost row of scutes.

On the plastron scutes we find in most species a spot of pigment for each member, but confusing secondary complexity often obscures the real pattern. In *Graptemys* some specimens

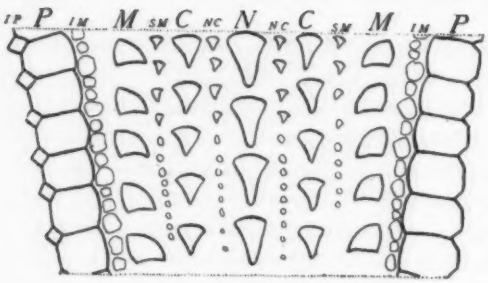

Fig. 55

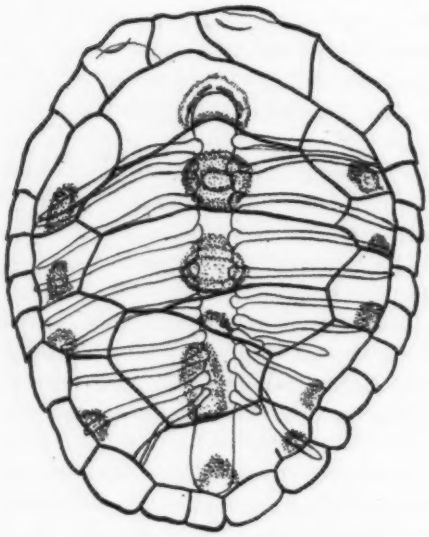

Fig. 56

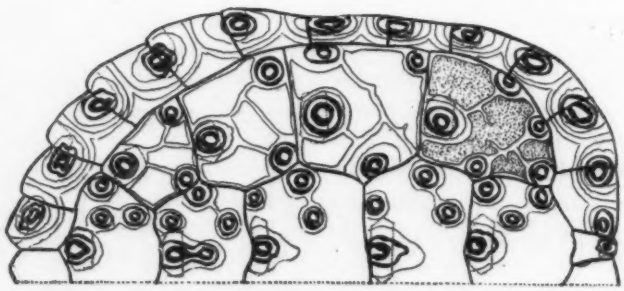

Fig. 57

Fig. 58

show a fairly complete set of ocellated spots, but others show almost colorless scutes. Adult specimens seldom show any sign of pigmentation on the plastron. The markings then are largely juvenile in character and are subject to very great individual variation that tends toward the total obliteration of the original color pattern. An examination of a number of specimens, however, shows that each of the plastron scutes may have its ocellated spot.

The most confusing markings of all are those that occupy the median portion of the plastron in juvenile specimens. A remarkable secondary complexity of pattern has arisen in this region that would be almost impossible of solution were it not for the close series of stages leading up from the simplest conditions. The simplest form of marking consists of small diamond-shaped patches of pigment at the inner angles of the plastron scutes. These spread along the margins of the scutes, form bands by splitting, and finally produce the complex lyriform pattern that one finds quite frequently. The position of the simplest marking is identical with that of the hypothetical interplastral scutes and probably once constituted the color-marking of this row of scutes.

All of the carapace and plastron markings have thus been accounted for as the growth centers of existing or lost scutes. This has been done in a species with a highly intricate color pattern and could be applied successfully, I believe, to any other species.

It should be mentioned that the color pattern of *Graptemys* reaches its highest development in specimens of the first year. This is the time when protective coloration is a necessity, as the carapace is not sufficiently ossified to furnish a protection. Old specimens retain scarcely a trace of the original pattern, only a very faint reticulation being visible.

It might be suggested that the ocellated spots of the Trionychidæ are vestiges of scutes long since lost. The general number and arrangement of these spots tends to bear out this suggestion.

Summary.

Palæontological and embryological evidence is at variance as to the origin and character of the neural and costal plates, but

observations point strongly to a periosteal origin of these structures, which means that they are in no sense dermal or the descendants of the original dermal carapace.

The testimony of comparative anatomy leads to the belief that the nuchal, procaudal, pygal and marginal plates are the remnants of a once more or less complete dermal carapace and that these plates formed the cores of scutes that must have had a more or less tubercular form. The keels of existing scutes represent these tubercules. The testimony of the tail-trunk of *Chelydra* indicates that there were originally seven primary rows of such scutes and that less prominent rows of scutes occupied the interspaces. These less prominent rows were gradually suppressed, first on the carapace and then on the plastron, beginning in the middle and proceeding laterally. Thus the first loss was the neuro-costal rows, second the supra-marginals, third the interplastrals, and fourth the inframarginals, which to-day persists normally in many primitive forms.

Accompanying the suppression of rows occurred a reduction in the number of scutes in the primary rows, and this reduction took a general antero-posterior direction. At the same time the rapid secondary growth of the neural spines and ribs caused the suppression of the corresponding dermal plates, leaving only the nuchal, procaudals, pygal and marginals in places where the internal skeletal portions failed to extend. Traces of the dermal armor in the mid-neural region have been found, however, in *Toxochelys* and *Graptemys*.

No correlation of abnormalities is to be expected in the neural and costal regions, since the scutes and plates of this region are entirely independent in origin, but in the marginal series, where the plates and scutes retain nearly their original connections, the correlation is perfect. In the procaudal region we find frequent correlations, but, that the correlation is not a necessary one, is shown by numerous uncorrelated abnormalities.

A study of the color-markings of *Graptemys* and *Chelydra* lends confirmation to all the above theories of the chelonian carapace and plastron, and at the same time serves to rationalize the patterns themselves.

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NOTE ON THE INFLUENCE OF SURFACE-EVAPORATION UPON THE DISTRIBUTION OF INFUSORIA.

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During the course of my experiments on the chemotaxis of *Paramœcium* and *Colpodium*[1] I was struck by the fact that in certain media the infusoria showed a remarkable attraction to the edge of the fluid under the cover-glass — these media were $N/25$ methyl alcohol, $N/50$ $CaCl_2$, $n/5{,}000$ NaOH and $N/5{,}000$ KOH. This is contrary to the usual behavior of the organisms, in other saline media, in sugar solutions and in the culture medium they avoid the edges of the film of water under the cover-glass and, if undisturbed, form a cluster in the middle of the film, or, if the cover-glass be supported at one end, a little away from the center, towards the supported end.[2]

Jensen attributed this to the increase in concentration at the edges of the film due to evaporation. Since infusoria appear to be very generally attracted by solutions of lower concentration they tend to congregate in the more dilute part of the film, that is, near the center. In considering the apparent contradiction to this rule displayed by the infusoria when immersed in the above-mentioned media it struck me that in all these cases we had the converse of Jensen's experiment — for these media tend to become more *dilute* at the edge of the film. Thus methyl alcohol evaporates more rapidly than water, so that a solution of methyl alcohol becomes more dilute as it stands exposed to the atmosphere — while solutions of highly hygroscopic substances $CaCl_2$, NaOH and KOH absorb water-vapor from the atmosphere and so also tend to become more dilute at the surface. In order to see whether this was the case I tried the effect of adding

[1] *Journal of Biological Chemistry*, January, 1906.

[2] Jensen, *Pflüger's Archiv. für ges. physiol.*, Vol. 53 (1893), p. 428.

different reagents to the culture-medium, in which the infusoria were suspended, upon their distribution under the cover-glass.

When a substance which evaporates more rapidly than water is dissolved in a watery medium containing salts in solution two contrary effects take place on evaporation — the solution becomes more dilute in respect to the more rapidly evaporating substance owing to its evaporation and more concentrated in respect to the salts owing to the evaporation of water. When the decrease in osmotic pressure due to the evaporation of the more rapidly evaporating substance is greater than the increase in osmotic pressure due to the increased concentration of the salts — then the total result is a decrease in the osmotic pressure. But if the initial concentration of the more rapidly evaporating substance be less than that necessary to bring about the above result then the increase in concentration of the salts will proceed more rapidly than the decrease in concentration of the volatile substance and the total result will be an increase in osmotic pressure at the surface of the fluid. Thus it was to be expected that while under the ordinary conditions of my experiments on *Chemotaxis* — when to a very small amount of culture a comparatively large amount of solution was added — methyl alcohol at a concentration of $N/25$ caused a dispersion of the infusoria to the edge of the cover-glass, when I added 1 c.c. of a solution of methyl alcohol to 5 c.c. of culture it was found necessary to bring the final concentration of the methyl alcohol up to $\frac{5}{6}N$ in order to get the first indication of a tendency to seek the edge — since in this latter case the salts were correspondingly less dilute. The solution of the volatile or of the water-absorbing substance in the culture medium having been made, drops of the mixture were placed on a slide under a cover-glass which was slightly raised at one end.

The following are the experimental results:

Methyl Alcohol. — 1 c.c. of a $5N$ solution of CH_3OH was added to 5 c.c. of the culture-medium in which numerous colpodia and paramœcia were suspended. Result at first uniform distribution of the infusoria under the cover-glass, in a few minutes, however, they began to congregate at the edges — especially at the edges farthest away from the supported end. In a short time all

parts of the film, except the edges, were free from infusoria, while dense clusters had been formed along the edges farthest from the supported end. When a $6N$ solution of methyl alcohol was used instead of a $5N$ solution the effect was more marked and appeared more quickly.

Ethyl Alcohol. — 1 c.c. of a $5N$ solution of C_2H_5OH was added to 5 c.c. of culture. The same effect was obtained as with methyl alcohol, but it was slightly less marked and took longer to appear.

Propyl Alcohol. — $\frac{5}{6}N$ C_3H_7OH was too toxic — the organisms being killed too soon to obtain any result — but in $\frac{3}{10}N$, which is about the strongest solution they will stand, there was no attraction to the edges of the film.

Ethyl Acetate. — 1 c.c. of a saturated aqueous solution of ethyl acetate was added to 5 c.c. culture. The organisms were at first uniformly distributed — they then showed marked attraction for the edges of the film, particularly for those edges most remote from the supported end — and in a few minutes all the infusoria were congregated at the edges at the shallower end of the film

Calcium Chloride.—1 c.c. of a $\frac{3}{5}N$ solution of $CaCl_2$ was added to 5 c.c. of the culture—attraction to the edges was observable for a short time. As in the other cases the edges farthest from the supported end became the most densely populated.

Potassium and Sodium Hydroxides.—In my experiments on *Chemotaxis* and under the conditions of those experiments I obtained a marked, though more or less transient, attraction to the edges of the film at a concentration of $N/5{,}000$.

All the above results hold equally for *Colpodium* and for *Paramœcium.*

In all cases the attraction to the edges finally disappeared — with ethyl acetate the effect passes off in about half an hour.

All these substances which I have found to cause aggregation of the infusoria at the edges of the film are substances the solutions of which tend to become more dilute on exposure to the atmosphere. Methyl alcohol, ethyl alcohol, and ethyl acetate evaporate more rapidly than water while $CaCl_2$, KOH and NaOH are well known absorbents of water-vapor. There is also a general correspondence between the rate at which the volatile substances evaporate and the intensity of the effect which they pro-

duce — thus methyl alcohol has a lower boiling-point than ethyl alcohol and it also causes a more marked attraction to the edges of the film.

The fact that in all these cases the attraction to the edges of the film disappears after a certain time may perhaps be due to the volatile substance having become so dilute in the film that the increase in concentration of the salts due to the evaporation of water now takes place more rapidly than the decrease in concentration of the volatile substance. While in the case of water-absorbing substances — they may have become so dilute that the water-absorption due to their presence in the solution now goes on less rapidly than the loss of water due to evaporation. It seems probable therefore that these phenomena of attraction to or repulsion from the edges of the film are in reality special cases of osmotaxis.

I have alluded to the fact, mentioned by Jensen in the paper to which I have referred, that under ordinary circumstances *Paramœcia* under a cover-glass supported at one end tend to collect in the middle of the film but towards the supported end. But when the substances which cause the infusoria to congregate at the edges of the film are added to the culture we obtain precisely the converse effect — the infusoria collect at the edges, *especially at the edges farthest from the supported end.* It thus appears probable that this is also an osmotactic phenomenon connected with surface dilution. One would be inclined to fancy that it was due to surface evaporation having more effect upon the concentration of the small bulk of liquid at the shallower end of the film than upon the greater bulk of liquid at the supported end — but the *ratio* of surface to volume is not greater at the shallower end than at the supported end. Dr. Loeb has suggested to me that it may be due to the fact that diffusion and therefore equalization of concentration is less rapid in capillary spaces than in the bulk of the liquid — that, in fact, the shallow end of the film may act in a manner analogous to Liebreich's "dead space."[1]

[1] *Zeitschrift für Physikalische Chemie*, Vol. V. (1890), p. 529 and Vol. VIII. (1891), p. 83.

Conclusions.

1. That whereas, under ordinary circumstances, infusoria tend to collect near the center of a film under a cover-glass, when methyl alcohol, ethyl alcohol, ethyl acetate, $CaCl_2$, KOH or NaOH are added in sufficient concentration to the culture-medium, this tendency is reversed and the organisms now gather at the edges of the film.

2. That this lends support to Jensen's explanation of the normal tendency of the infusoria to gather near the center of the film, namely, that it is a special case of osmotaxis brought about by surface-evaporation.

HISTOGENESIS IN INSECT DEVELOPMENT, AND CELL SPECIFICITY.

VERNON L. KELLOGG.

In the development (ontogeny) of insects with complete metamorphosis the imaginal antennæ, mouth-parts, legs and wings are produced from small buds, or histoblasts, in the larval derm. These histoblasts or imaginal buds arise by the shallow or deep invagination (one for each histoblast) of small regions of the larval cellular skin layer including originally, in each case, only comparatively few derm cells. The position of these invaginations, and therefore the participation of derm cells in the future leg or wing development, seems to be determined wholly with reference to the future imaginal organ, and not at all with reference to any difference in degree of differentiation among the cells of the larval derm. The wing-buds arise from the latero-dorsal regions of the meso- and meta-thoracic segments, the leg-buds from the latero-ventral regions of each of the three thoracic segments.

The larval derm is certainly not to be looked on as composed of wholly undifferentiated embryonic cells. These derm cells make up a definite organ, or part, of the larval body, with definitive position and particular functions. All these cells, and no others in the body, secrete[1] chitin; some of them secrete noxious fluids, ill-smelling, acrid, poisonous. Many of them, perhaps all, secrete moulting fluid at the times of the regular larval moults; many are specially sensitive, many bear sense-hairs or papillæ.

The invagination and beginning development of small parts of this derm, in a wingless and legless larva of a fly or honeybee or of some other specialized insect, may occur in a very early larval stage (in some cases, indeed, indications of the future histoblasts are apparent in just-hatched larvæ), but in most cases the invagination does not appear until a certain part of the free larval life has been lived. That is, the larval derm has for awhile

[1] The chitin secreting capacity of the anterior and posterior thirds of the insect alimentary canal is due to their deeply invaginated derm.

subserved only, and has subserved fully, the functions of skin, and its cells have certainly attained whatever differentiation they need for the performance of these functions. It is far and away a long cry back to the embryonic, undifferentiated, the non-specific condition. But apparently any part or region of this derm which may, by its position, be the region or part needed to develop an antenna, a wing, a leg, male clasper, female ovipositor, or a sting, can respond to the need, and by invagination (for protection's sake), rapid growth and proliferation of cells, quick differentiation and arrangement, and final evagination (at the time of the last larval moulting, *i. e.*, pupation) produce the needed organ. This organ may be tubular and segmented, and the segments may be similar (antennæ); or dissimilar (leg); or it may be a great flattened sac, supported by tubular skeletal ribs and covered by a million and more tiny other striated, pigment-bearing, flattened sacs (the butterfly's wing with its scales); or it may be the exquisite mechanism of the bee's sting.

This histogenesis of the imaginal parts of the fly, the bee, and the moth is, to my mind, an extremely suggestive phenomenon when considered in the light of its relation to the theories of cell-specificity or cell-non-specificity. Quite as positively as the more familiar cases of restorative regeneration (legs, eye-lenses, tails and what not of various vertebrates), does this radical histogenesis, common to the ontogeny of all insects with complete metamorphosis, make it impossible to limit the germ-plasm to the germ-cells. It stands strongly opposed to any theory of absolute cell-differentiation or cell-specificity.

STANFORD UNIVERSITY, CALIF.

ONTOGENY OF THE ANNULUS VENTRALIS.

E. A. ANDREWS.

In crayfish of the genus *Cambarus* there is a sperm receptacle in the female which has been known as the *Annulus ventralis* and made use of as a specific character in systematic works.

Nothing has been known of its mode of development beyond the brief mention by Mary Steele[1] who figured the external views of the annulus in specimens of *C. gracilis*, 20, 22, 27.5, 30, 35, 36, 50 and 60 mm. in length. Of these the latter four were probably adults and the former four immature young. These figures show an increasing complexity of external sculpturing and an increase in relative longitudinal diameter; however, as they were made incidentally and without reference to the use or internal structure of the organ they are necessarily insufficient. While the annulus is a necessary reproductive organ in *Cambarus* it is also a new organ in the history of crayfish since it is found only in the most specialized form, *Cambarus*. It seemed, therefore, of interest to find out just how and when the organ develops in the ontogeny of *Cambarus*.

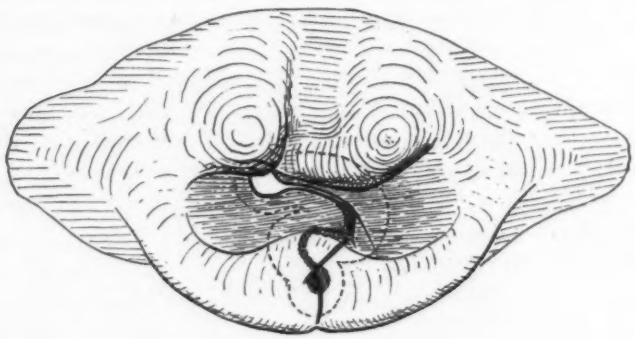

FIG. 1.

The following account of the origin and growth of the annulus refers almost exclusively to *Cambarus affinis* reared in the labo-

[1] *Univ. of Cin. Bulletin*, X., 1902.

ratory. In large adults, 100 mm. long, the annulus in this species has the appearance outlined in Fig. 1, which is enlarged about twelve diameters. It is a transversely elongated plate, part of the shell, with a central depressed area bounded behind by a cross ridge and in front by two high tubercles or tuberosities. Across the depressed area runs a zigzag line which is in reality a closed suture whence a slit leads inward to a curved tube represented by the thick shaded line. The suture and the curved tube both open out on one side into the depressed area by an orifice partly under one of the tuberosities. The walls of the tube are thick chitinous continuations of the shell, as indicated by the broken lines. Underlying this chitinous mass is the epidermis which forms it and which was found to be folded in as a bent groove. A comparative study of annuli in several species showed that while the external sculpturing is various, the presence of a curved epidermal groove is constant and that, morphologically, this sperm receptacle is a bent epidermal pocket lined by chitin and opening to the exterior by a more or less closed slit.

The position of the annulus, as seen in Fig. 2 which is enlarged one and a half diameters, is on the ventral surface of the thorax

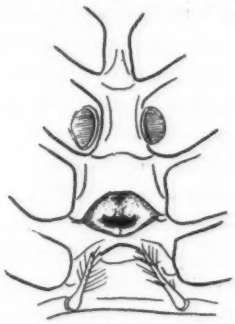

FIG. 2.

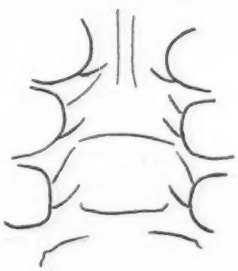

FIG. 3.

between the bases of the fourth pair of legs. The sternal surface behind it is elevated as a rounded knob that may be of importance in discharging the receptacle. Projecting forward towards this are the short pleopods of the first abdominal somite. Anteriorly, on the bases of the third pair of legs, are the large elliptical openings of the oviducts whence the eggs when laid pass back over the annulus.

In using the annulus as a sperm receptacle the male passes the sperm into the orifice and thence into the posterior part of the tube. The anterior part of the organ, the orifice itself and the following transverse part, which may be called the vestibule, is filled not with sperm but with a cement that protects the sperm from the water. Eventually the sperm issues out through the more posterior part of the suture at the right time to meet the eggs.

When the young *C. affinis* hatches from the egg there is no annulus present. The ventral surface (Fig. 3), multiplied fifty diameters, shows no specialization of the wide level area between the bases of the fourth legs. The sexes are not yet distinguishable and the first abdominal appendages are in all cases but very faintly indicated by slight elevations. Here under a higher power the epidermis was seen to be specialized as a group of nuclei, over which the cuticle was elevated as a slight protuberance in the region that is later to grow out as the first abdominal appendage.

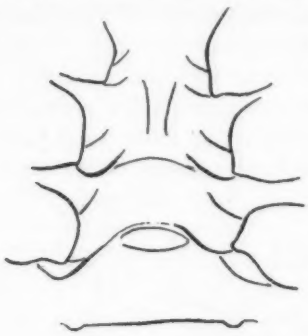

FIG. 4.

After this larva sheds its shell and passes into a second stage the sternal surface is larger (Fig. 4), and the first abdominal appendages are somewhat more protuberant. There is, however, no annulus and no external signs of sexual differences. Between the bases of the fifth legs there is now a transverse elevation of the sternum, which will become the prominent tubercle posterior to the annulus in the adult.

After a second moult the larva in the third stage has a much larger sternal area and for the first time sexual openings and the beginning of the annulus. In the female (Fig. 5), the sternum between the fourth legs is divided by a cross-line into an anterior part articulating with the legs, and a broad posterior region that is, however, markedly short. On the middle of this plate and at its posterior edge is a slight depression or groove destined to become the receptacle for sperm in the adult. In the male the same differentiation of a posterior sternal area is found, but the

plate lacks any groove and henceforth remains without any special development. The female is also recognizable by the appearance of the short, curved cuticular ridge on the base of the third leg, each side of the body, which is to be the median edge of the future orifice of the oviduct. From this superficial ridge a cylindrical epithelial tube, the oviduct, leads into the interior of the body. The first abdominal appendages, or pleopods, are blunt papillæ and apparently the same in both sexes, though sometimes in the male they seem more pointed and possibly longer.

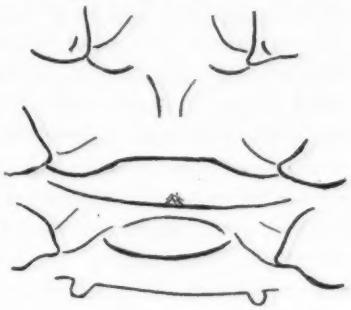

FIG. 5.

The annulus is thus a narrow transverse part of the sternum and since its posterior edge projects and overhangs somewhat it might be regarded as a sort of transverse fold. Its surface is entirely flat and simple as compared with the complex adult surface, Fig. 1, except for the slight median depression. Looking at the shell only, Fig. 6 enlarged two hundred diameters, this depression is a wide shallow groove indicated by converging wrinkles in the cuticle on each side the middle line of the animal and ex-

FIG. 6.

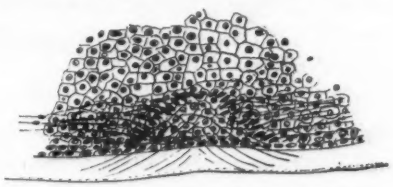

FIG. 7.

tending forward but a slight distance from the posterior edge of the annulus. The posterior edge of the annulus is rounded and protuberant and the groove to some extent extends over this posterior face of the annulus and cannot well be all seen at once from a ventral point of view. While this groove in the shell differs in different specimens it is in no way an artificial result of methods

of preservation but is based upon a special arrangement of the epidermis, which, Fig. 7 enlarged two hundred diameters, is a single layer of polygonal cells, close under the cuticle. This layer dips down under the groove and on the side of the groove, where seen in profile, the cells are elongated and continued as fine fibers that connect with connective tissue cells and with a membrane separating the epidermis from the large blood sinus just above it. In the figure these cells are represented in black as seen in optical section and also represented as seen in surface view upon another focus. Posteriorly the epidermis had shrunken away from the cuticle, in preparation, and left a wide artificial space. In an actual cross section of this region the groove is found to be just below the posterior part of the ganglion, Fig. 8, that supplies the fifth pair of legs, so that one might argue that the annulus belonged to the last thoracic somite while lying in the penultimate one. As seen in Fig. 8, which is enlarged two hundred

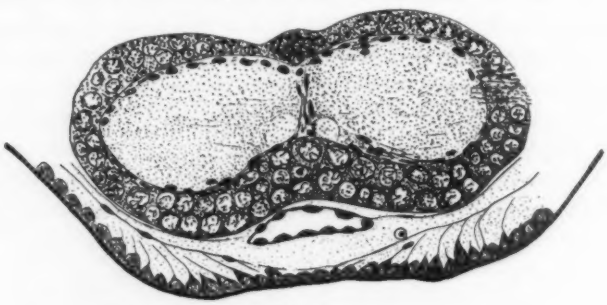

FIG. 8.

diameters, the epidermis cells fit together as polygons only at their outer ends next the shell while their inner ends taper off as fibers which diverge right and left of the central part of the groove. The epidermal cells are thus stretched out laterally and arranged on each side with reference to the superficial median groove that is to become the sperm receptacle.

Enclosing the epidermal cells is a coagulum of blood partly separated by a membrane from the blood space beneath the nerve cord. In this space lies the large median ventral artery that anterior to this section connects through the nerve cord with the

descending artery from the heart, between the fourth and fifth ventral thoracic ganglia.

Young crayfish in the above third stage are about 7–8 mm. long. In the fourth stage they are usually 11 mm. long. By this time the males and females differ noticeably in the lengths of the first pleopods. In the female, Fig. 9, they are still very small but larger than in the previous stage, this figure being enlarged

FIG. 9.

FIG. 10.

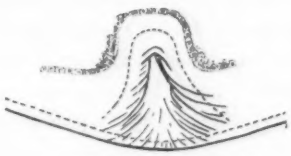

FIG. 11.

but thirteen, and Fig. 5, fifty diameters. In the male, Fig. 10, on the same scale, the pleopods are long, slender, but simple cylinders pointing toward one another. These two figures also show the increasing longitudinal diameter of the anmulus in the female and its simple form in the male, as well as the reproductive openings upon the fifth legs in the male and the third legs in the female.

In these females 11 mm. long the median groove of the annulus is much more evident than at first, but is still a simple groove as shown in Fig. 11 enlarged 200 diameters. The walls of the folded in and thickening shell that bound the deep and narrow groove are indicated by the broken line. In this preparation the epidermis had shrunken far away from the shell and is indicated in optical section to show its invaginated state where it came under the cuticular groove. The groove seems to grow forward from the posterior face of the annulus and becomes more narrow and closed in anteriorly. At the anterior tip the groove was partly overhung by a cross fold, or tended to burrow under the surface as a short *cul de sac*.

In different females, however, the groove had different lengths and more or less of this covering-over of the front end. In a specimen 10 mm. long the groove was longer and more narrow than the one above figured. In others of 11 mm. the anterior

end was crossed by a sharp fold and the *cul de sac* was more pronounced. In some the posterior face of the annulus, which projected and overhung considerably, bore a curved transverse ridge strongly suggesting the posterior rim of the adult annulus, Fig. 1. In a large female of 13 mm. the groove was as in Fig. 11, though a little longer.

The single layer of epidermal cells under the shell was still visible as a series of polygons as in the third stage. While the annulus itself showed no setæ upon it in any stage the transverse ridge of the sternum behind it bore scattered over it some four or five sharp setæ in the third stage, about ten in the fourth and twenty in the fifth.

The fifth stage includes individuals 15–18 mm. long, and in them marked changes had taken place in the annulus, but before describing these we will refer to a few observations made upon the developing annulus in an early stage of another species, *C. Clarkii*, from New Orleans. Here also the young reared in the laboratory had no annulus in the first and second stages, which were about five and six millimeters long, and they also showed no external sexual differences. Young of this species were seen by Hagen,[1] when .3 inch long and still on the abdomen of the female; in which the females had no pleopods upon the first abdominal somite, but showed the openings of the oviducts, while the males had the first pleopods as little knobs longer than broad and turning inward. Later Faxon[2] stated that the young of this species from under the abdomen of the parent had the general form of the adult when 7 mm. long. Evidently both authors saw the third stage only.

Eggs hatched April 21 reached the third stage June 1, but no observations were made upon the annulus till they had passed into a later stage, June 19, and were 11 mm. long. In this condition the male and female were much like specimens of *C. affinis* of that length, but the first pleopods in the female were much smaller and in the male noticeably shorter than in *C. affinis*.

The groove on the annulus of the female now formed a deep, closed in cavity, Fig. 12, which is enlarged to the same extent as

[1] "Monograph N. A. Astacidæ," Harvard, 1870.

[2] "Revision of the Astacidæ," Harvard, 1885.

Fig. 11. In *C. Clarkii* the groove has closed, leaving a suture, represented by the black line, above an internal cavity, represented in white and this is bounded on the sides and bottom by the infolded cuticle, represented in dotted lines. At the posterior end the groove passes around the edge of the annulus and seems

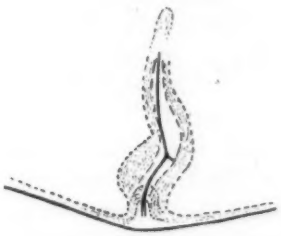

FIG. 12.

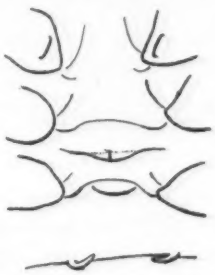

FIG. 13.

to be still open there. At the front end the groove pushed forward as a blind growth beneath the surface. Thus in *C. Clarkii* the receptacle though probably formed at the same stage and in the same way as in *C. affinis*, yet advances more rapidly, so that in the young 11 mm. long it is comparable, in its closed up condition, to *C. affinis* when 21 mm. long and in the sixth stage.

Returning to *C. affinis* in the fifth stage, 15–18 mm. long, the general appearance, Fig. 13, enlarged 13 diameters, shows an increase in size of the animal and a great growth of the first pleopods of the female which are now much longer than in the female of the fourth stage and nearly as long as the male pleopods of that stage, Fig. 10.

The receptacle itself is quite diverse in different individuals. In all there is added onto the median groove two elevations or folds which tend to cover over the anterior end of the groove. The groove itself is bent on one side more or less and the overhanging folds are more or less developed. In the specimen shown in Fig. 14 as enlarged 200 diameters the groove bends to one side and passes in under a marked flap or fold that grows over the tip of the groove. This lateral fold may be called the "hood" to distinguish it from the longer fold which passes along the opposite side of the groove and tends to overhang it.

This second fold is slightly developed in this case. In another specimen, Fig. 15, the groove bent very far to one side and the anterior part was concealed under the second fold. We will call this second fold the "transverse" fold, since it ultimately lies

FIG. 14.

FIG. 15.

more nearly across the median plane. Both folds are oblique, the "hood" and the "transverse" fold being about at right angles where the latter passes under the former. In Fig. 15 not only is much of the groove overhung by the transverse fold, but the entrance to the groove in under that fold is cut off, near the hood, by a short posterior fold that runs parallel to the transverse fold as it emerges from under the hood.

The cases of unusual bending of the groove to one side suggest the state of things found in the adults of *C. immunis* and *C. Bartoni* where the receptacle is more transversely placed than in *C. affinis*.

The specimen last figured was about to shed and the delicate new shell within the one here figured was more like that figured below for the next stage.

In another case the shell cast off by a larva going into the sixth stage had the groove but slightly bent, Fig. 16, and the

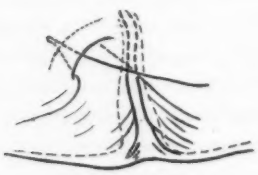

FIG. 16.

FIG. 17.

transverse fold was less oblique. As there was no fold opposing the transverse fold an object could have passed under its pos-

terior edge and thence to the anterior part of the groove. The receptacle was now being formed of three different parts: the original median groove, the two oblique folds. The former remains as the posterior part of the adult sperm tube and the latter help to make the orifice and the vestibule, or anterior part of the adult receptacle.

In all these cases the groove bends more or less to one side, and in only one case observed was the bending to the observer's right, which is the left of the animal. In this exceptional animal, Fig. 17, not only does the groove bend to the right, but the hood is on the right and the transverse fold and the posterior opposing fold run at right angles to their usual course. Comparison of Fig. 17 with Fig. 15, shows that they are, in the main, mirror-images of one another. Each reversed, as seen through the paper, would have the symmetry of the other.

This reversal of symmetry in the receptacles of some young is the first visible expression of the peculiar dimorphism of the annulus in the adults of this and other species. While many of the adults have the orifice upon the right side of the median plane others have it upon the left and in all respects these two forms of annulus are mirror-images of one another. Both forms are used as sperm receptacles. Those with the orifice upon the right, Fig. 1, are more common; in one lot of 41 females, 38 had these right-handed annuli.

The characteristic tuberosities of the adult grow out in later larval life and they also are right- and left-handed in the following way: while, usually, as in the above figure, the tuberosity upon the left of the animal sends a ridge under the right tuberosity, in left-handed adult annuli the behavior of the tuberosities is the reverse. In the production of the two symmetrical adult forms there is thus the harmonious development of the groove, folds and tuberosities at different periods and from several areas of the epidermis.

In July the larva may pass into a sixth stage 21 mm. long. On the ventral side, Fig. 18, enlarged thirteen diameters, there is a noticeable increase in the longitudinal diameter of the annulus and in the length of the first pleopods which are now longer than those of the male of stage four, Fig. 10, though closely applied

along the sternum and, apparently, of no use as yet. The annulus, however, has acquired a rather mature appearance. When enlarged fifty diameters, Fig. 19, it shows toward the center two gentle elevations to represent the future tuberosities and posteriorly a transverse curved rim like that of the adult. The whole plate is still narrow from before back but much less so than in previous stages. The suture of the receptacle is indicated by the zig-zag line and its lateral walls by the broken lines. The former represents: anteriorly on the left the former edge of the hood; posteriorly the closed up groove; and in its middle course the transverse fold.

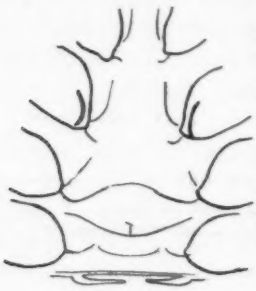

FIG. 18.

Enlarged 200 diameters, Figs. 20 and 21, the receptacle presents varieties of development in different individuals. The groove itself may be open posteriorly as in Fig. 20 or quite closed

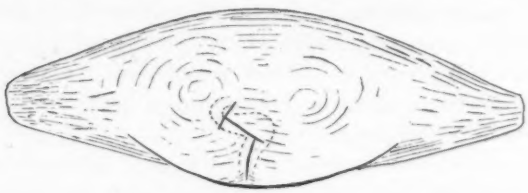

FIG. 19.

up as in Fig. 21. The anterior part of the groove bends far to one side to end under the hood at the right end of the transverse fold. This part of the groove may be still accessible by passing in under the transverse fold, Fig. 20, or it may be quite shut off by a posterior fold growing along against the posterior edge of the transverse fold as in Fig. 21. Fig. 20 is not much advanced beyond the preceding stage, Figs. 15 and 16, the open groove, the hood and the transverse fold are larger and the invaginated shell is more extensive, as indicated by the broken lines. But in other cases, Fig. 21, the deeply buried groove connects with the surface only by oblique planes that come to the surface as the three

successive parts of a zig-zag suture. The inner course of the groove is more sinuous than before in correspondence with the increased bending of the invaginated shell that forms its walls.

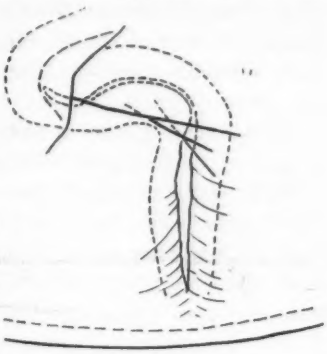

FIG. 20.

The chief features of the adult sperm pocket are thus present except that the anterior orifice seems more closed in some individuals.

Though in some cases it seemed as if the groove itself did not bend to one side to end under the hood, but rather that the transverse fold left a passage from the groove to the hood, the true state of the epidermal groove upon and in which the shell groove is formed was seen when the shell was pulled off. Then, in Fig. 22, enlarged 200 diameters, there is a deep, bent furrow with high sides formed of long epidermal cells, as somewhat diagrammatically represented in this camera lucida sketch of an optical section

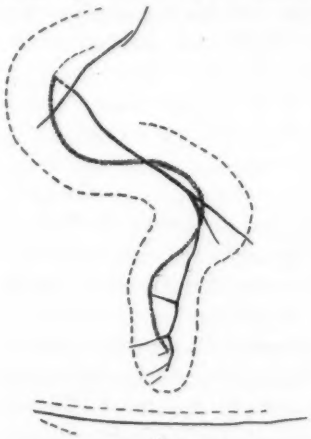

FIG. 21.

FIG. 22.

of the epidermal part of the receptacle. In this specimen the shell was easily removed since a new shell was in process of formation to

line the groove. This made the groove more distinct than otherwise is the case. The groove passes forward from the posterior edge of the annulus and then bends to one side much as does the bottom of the shell groove seen as a shaded line in Fig. 21. In each individual, however, the amount of bending seems to be different.

Such a bent groove is very like the simple bent epidermal groove of *C. Clarkii*, which is one of the less specialized species.

While previous experience had shown that larvæ after living eleven days in the above sixth stage might turn into a seventh stage 29 mm. long in the middle of July, no observations were made upon the above larvæ 21 mm. long till October 3, 1904, when they had turned into individuals 25–53 mm. long. Those 25–35 mm. agreed with one another in the development of the annulus and probably represented larvæ that from lack of enough food had remained in the seventh stage while the female, 53 mm. long, belonged to some later stage. In this laboratory it has been found that crayfish of that latter size have their sexual instincts developed in the fall and females 52 and 53 mm. long, being then supplied with sperm by males of the same or even smaller sizes, laid good eggs the following spring when not quite one year old. Thus the above female was probably mature in instincts and in external sexual organs when examined.

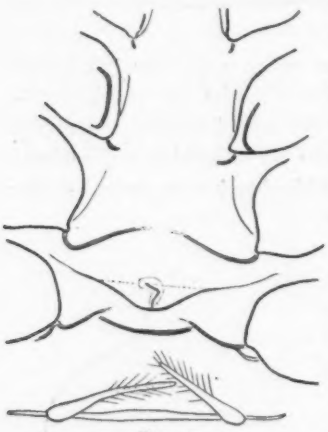

FIG. 23.

First, however, taking up the specimens 25–35 mm. long which were all essentially alike except in size, one 35 mm. long enlarged 13 diameters, Fig. 23, presents a noticeable growth of the first pleopods which are now turned forward and sparsely set with hairs as in the adult, and also much increase in the relative importance of the curved ridge bounding the mouth of the oviduct opening on the third leg. As an opaque object the annulus now

showed very definite tuberosities separate from the hood and the transverse fold and the tuberosity upon one side of the animal extended over the median line, in carrying out the asymmetry of the adult. The general proportions of the various folds and thickenings of the shell now approximated the adult condition.

The receptacle had not materially changed from the previous stage, Fig. 21, but its invaginated shell walls were greatly thickened and laminated, Fig. 24, enlarged like Fig. 21. It would appear that with the dropping of the bottom of the invaginated

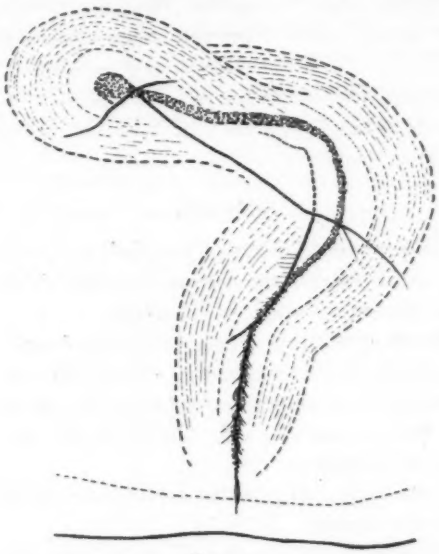

FIG. 24.

groove away from the surface its sides have closed in to form a narrow crevice which comes to the surface as a suture line. Then by the bending of the bottom of the invagination more than its surface suture line these crevices are made into curved oblique planes. The hood and the transverse fold and the posterior opposing fold are intimately associated with the bending of the original groove to one side and then the sinking of that lateral bend away from the surface and diagonally forward.

The last young to be considered, the larva, 53 mm. in length

and sexually mature, has an annulus very much like that of the full-grown adults, but it is still very small, as is seen on comparing Fig. 25 with Fig. 1, both enlarged 12 diameters. Still the tuberosities do not yet overhang the transverse depression enough to conceal the hood and the transverse fold, nor are the transverse depression and the posterior rim as well developed as later. The receptacle itself is much like that in Fig. 24, but its orifice is more perfect, though not yet as patent as in the large specimens, Fig. 1. This young annulus still lacks the posterior enlargement of the sperm tube, indicated in Fig. 1, and the complexity of bending of the tube is less, nevertheless it functions as a sperm receptacle.

FIG. 25.

SUMMARY.

The specialized sternal plate of the shell of *Cambarus*, which in the adult female bears the sperm-receptacle, is first differentiated in the third larval stage, in *C. affinis*.

The epidermis under this plate grows inward and outward in special areas to form the receptacle. First there is formed an open median groove that then bends to one side, sinks away from the surface and becomes closed. In *C. Clarkii* also one stage shows a like development.

Next definite folds overgrow the anterior end of the groove as it sinks from the surface.

Later elevations and depressions complete the external sculpturing of the annulus.

The anterior part of the groove, with the accompanying folds, form the orifice and vestibule of the sperm receptacle, while the posterior part of the groove forms that part of the receptacle in which the sperm is stored.

A functionally complete annulus is made within five months, but subsequently it becomes more complex. Comparative study of the adults of several species has shown that in *Cambarus* the essential part of the annulus, as a sperm receptacle, is a curved pocket; the above facts indicate that in *Cambarus* in general this pocket arises as an open epidermal groove.

The right or left handed symmetry of different adult annuli is first visible in the fifth larval stage when the median groove has bent to the right or to the left and the accompanying folds have a reversed position in the two cases. Later other outgrowths may also harmonize with the groove and folds to complete the two adult forms; mirror-images of one another.

While the sperm receptacle is a necessary organ in *Cambarus* it is phylogenetically a new one since *Cambarus* is the most specialized genus of crayfish and other genera have no receptacle. In *C. affinis* this new organ is not seen till the individual has reached a third stage after leaving the egg.

Besides beginning late in ontogeny this new sperm receptacle is variable in all its stages of growth. In its early and simple state in the larva it resembles the adult receptacle of a less specialized species, *C. Clarkii*.

In some of its variations of lateral bendings in early larval stages it suggests adult conditions in other species.

BALTIMORE,
December 12, 1905.